

CHEMILUMINESCENT SUBSTRATES FOR  
ACETYLCHOLINESTERASE AND ALKALINE PHOSPHATASE

FINAL REPORT

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## ABSTRACT

A new type of adamantyl-stabilized 1,2-dioxetane which incorporates a benzothiazole group has been developed. Chemical and enzymatic triggering of these dioxetanes in aqueous solutions provides efficient chemiluminescence. The quantum yield for luminescence is enhanced by as much as 5,000-fold compared to similar dioxetanes not substituted with the benzothiazole group. The synthesis of a phosphate-substituted dioxetane and its triggering with alkaline phosphatase is described. The development of chemiluminescent substrates for  $\alpha$ -chymotrypsin,  $\beta$ -glucuronidase and aryl sulfatase is reported.

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## SUMMARY OF RESEARCH

### **1. Efficient Chemiluminescence from an Adamantyl-Stabilized Phosphate Dioxetane in Aqueous Solution**

#### Statement of Problem

The primary objective of this research was to develop thermally stable dioxetanes which could be chemically and enzymatically triggered to provide efficient chemiluminescence in aqueous solution.

It had been found previously that triggering of dioxetane **1a** and related silyloxy-substituted dioxetanes with fluoride in DMSO provides intense blue chemiluminescence with a quantum yield of 0.25 and chemiexcitation yield of 0.57, one of the highest singlet chemiexcitation yields reported for a dioxetane.<sup>1</sup> In contrast, chemical triggering of this and related dioxetanes with various reagents in aqueous solutions resulted in extremely low levels of luminescence ( $8 \times 10^{-6}$ ) due to the non-fluorescent properties of the carbonyl cleavage product (methyl m-hydroxybenzoate) in protic media. Similarly, alkaline phosphatase-catalyzed dephosphorylation of dioxetane **1b** also produced chemiluminescence with low efficiency (Scheme 1).<sup>2</sup>

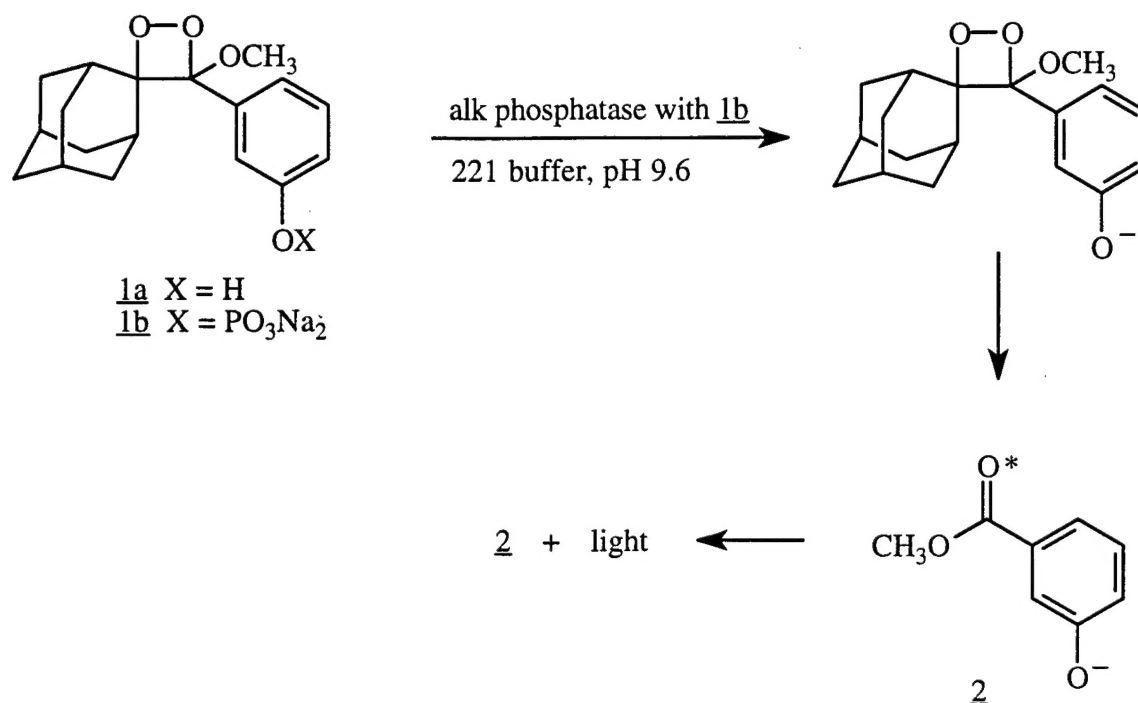
We have previously enhanced the chemiluminescence quantum yield of these dioxetanes in aqueous solutions through inter- and intramolecular energy transfer processes.<sup>2</sup> The results described in this report concern an alternative approach in which the structure of the ester cleavage product was modified so that it is inherently fluorescent in water. In designing the dioxetanes to be investigated, we kept in mind our earlier work which had shown that it was important to retain the meta-hydroxy phenyl moiety for efficient generation of the excited state.

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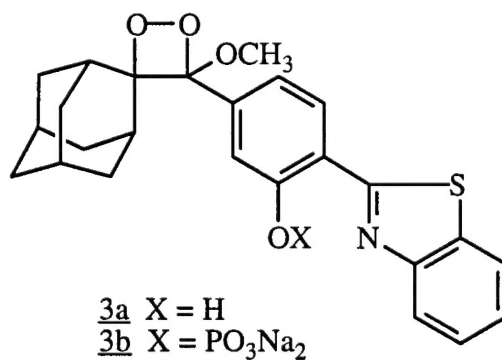
<sup>1</sup>A. P. Schaap, T. S. Chen, R. DeSilva, B. P. Giri, and R. S. Handley, *Tetrahedron Lett.*, 1155 (1987).

<sup>2</sup>A. P. Schaap, H. Akhavan, and L. J. Romano, *Clinical Chem.*, 35, 1863 (1989).

Scheme 1. Alkaline Phosphatase-Catalyzed Decomposition of Dioxetane 1b



This project therefore involved the synthesis and investigation of a new type of dioxetane 3 which incorporated both benzothiazol and meta-hydroxy phenyl groups. This research has shown that dioxetanes 3a and 3b are chemically and enzymatically triggered in aqueous solution to generate efficient chemiluminescence.



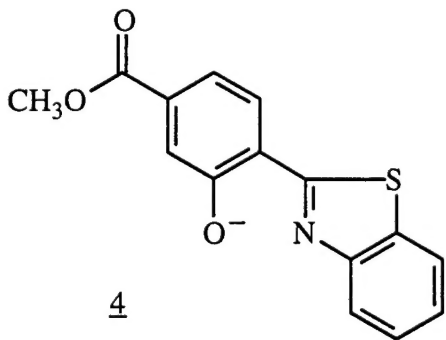
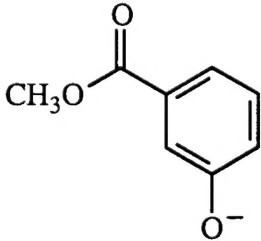


## Summary of Results

### A. Fluorescence Quantum Yield of 2-(4'-Carbomethoxy-2'-hydroxyphenyl)benzothiazole (4)

The fluorescent properties of the light-emitting product 4 produced by the chemical and enzymatic triggering of dioxetanes 3a and 3b was first investigated. This previously unknown ester was found to be highly fluorescent, both in aprotic and protic media (Table 1). In contrast, the fluorescence quantum yield of the phenolate ion of methyl 3-hydroxybenzoate (2) in DMSO is 0.44, while in water the value is too small to be measured. The fluorescence excitation and emission spectra of 4 in 0.75 M 2-amino-2-methyl-1-propanol (221) buffer pH 9.6 are shown in Figure 1.

Table 1. Fluorescence Quantum Yields of 2 and 4 in DMSO and Aqueous Solution

Compound	$\Phi_F$ / Conditions		
	F <sup>-</sup> / DMSO	aq. NaOH	221 buffer, pH 9.6
 <u>4</u>	0.75	0.62	0.59
 <u>2</u>	0.44	—	—

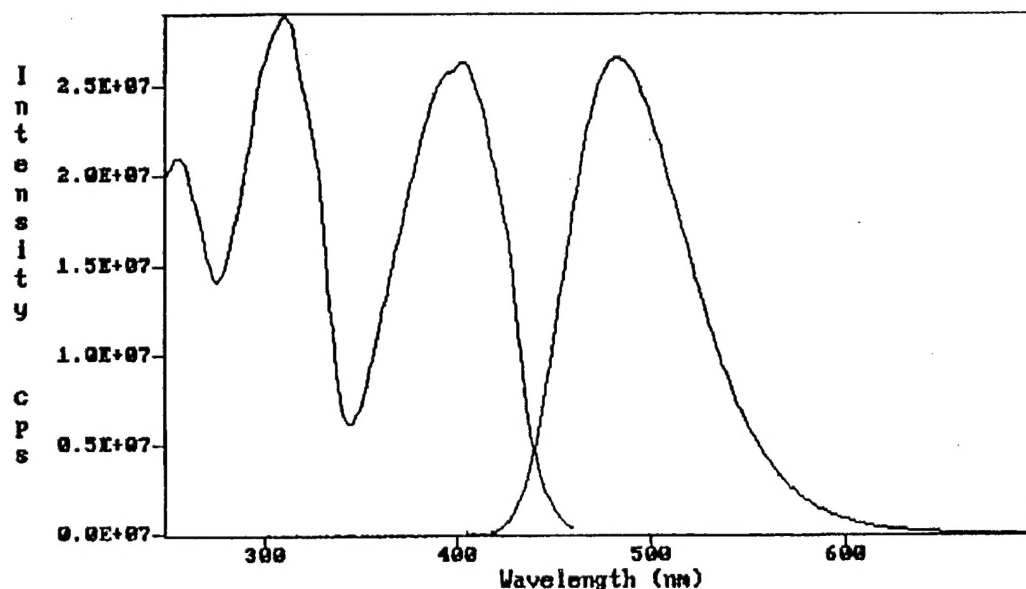


Figure 1. Fluorescence excitation ( $\lambda=311, 402$  nm) and emission ( $\lambda=483$  nm) spectra of the anion of 2-(4'-carbomethoxy-2'-hydroxyphenyl)benzothiazole (**4**) in 0.75 M 2-amino-2-methyl-1-propanol buffer pH 9.6 at room temperature.

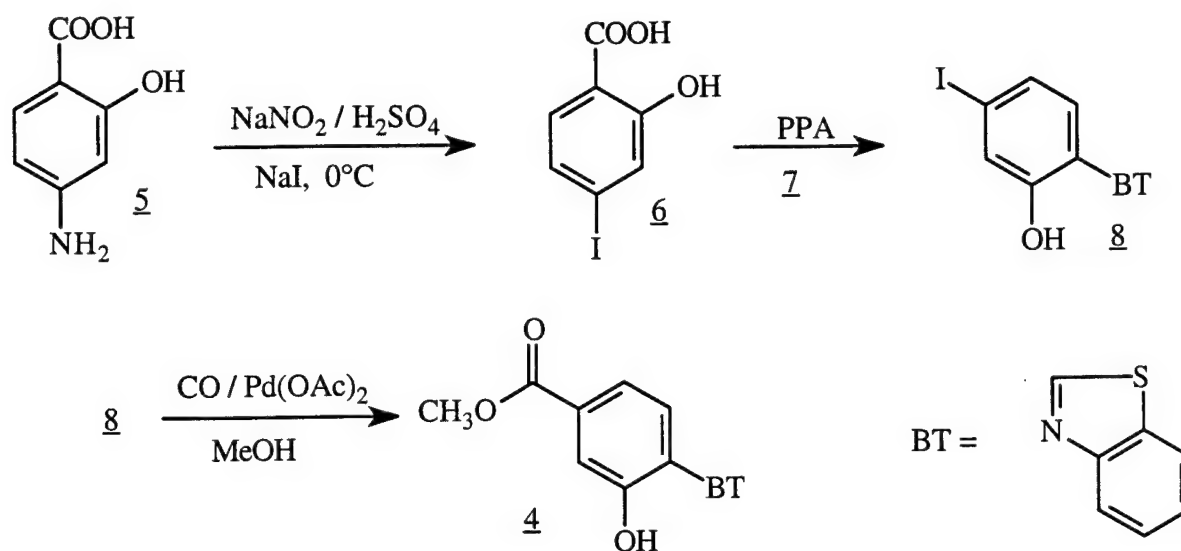
#### B. Synthesis of Dioxetanes **3a** and **3b**

Dioxetanes **3a** and **3b** were synthesized by the reaction sequences shown in Schemes 2 and 3. 4-Aminosalicyclic acid (**5**) was converted to 4-iodosalicylic acid (**6**) by diazotization with  $\text{NaNO}_2$  and reaction with  $\text{NaI}$ . Condensation of **6** with 2-aminothiophenol (**7**) in polyphosphoric acid afforded the benzothiazol-substituted phenol **8**. Palladium (0) catalyzed methoxycarbonylation of **8** gave the highly fluorescent methyl ester **4**.

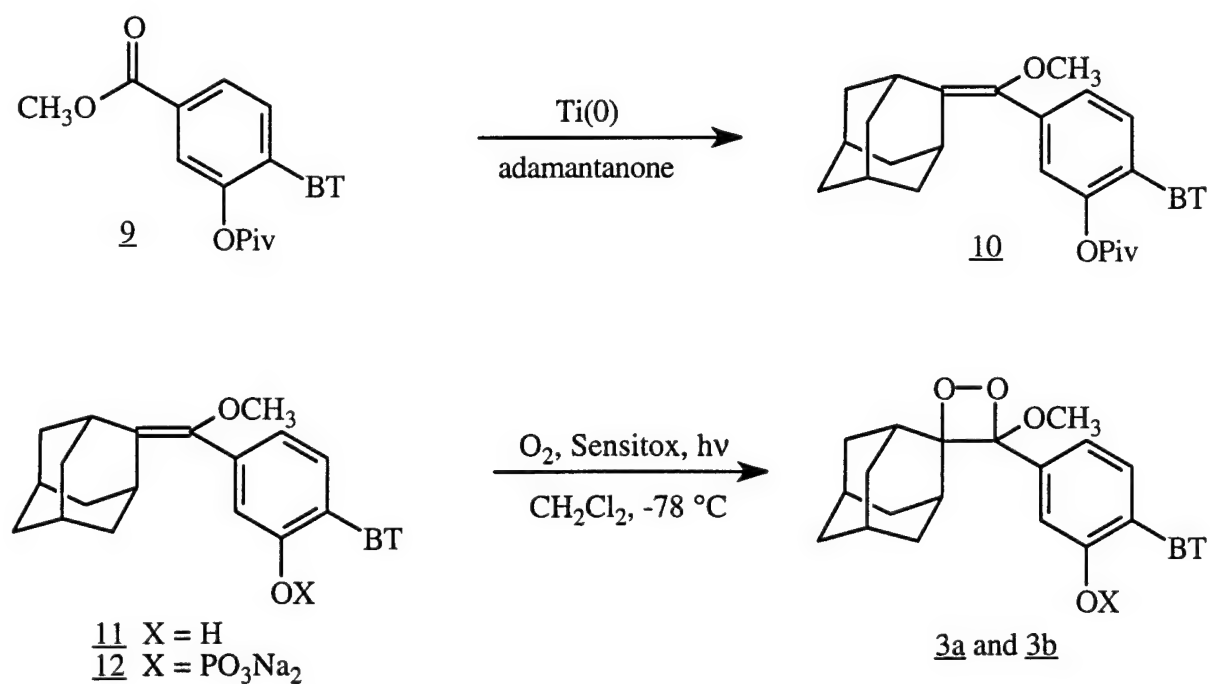
The alkene precursors to dioxetanes **3a** and **3b** were prepared by first protecting the hydroxy group in **4** with pivaloyl chloride ( $\text{PivCl}$ ) to give **9** which was subsequently reacted with adamantanone in the presence of  $\text{Ti(O)}$  to yield alkene **10**. Deprotection of **10** with tetramethylammonium hydroxide in methanol gave the hydroxy alkene **11** which was phosphorylated to form phosphate alkene **12**.

Dioxetanes **3a** and **3b** were prepared by photooxygenation of alkenes **11** and **12** respectively with a 400 W high pressure sodium lamp and polymer-bound Rose Bengal (Sensitox). The dioxetanes were fully characterized by NMR spectroscopy.

Scheme 2. Synthesis of Benzothiazoyl-Substituted Ester **4**



Scheme 3. Synthesis of Dioxetanes **3a** and **3b**



### C. Chemical and Enzymatic Triggering of Dioxetanes **3a** and **3b** in Aqueous Solution

Treatment of dioxetane **3a** in aqueous basis buffer produces the unstable phenoxide form of the dioxetane **13**. Subsequent decomposition of this species produces the singlet excited state of ester **4** which fluoresces to generate the observed chemiluminescence as shown in Scheme 4. Triggering experiments with **3a** were carried out in 0.1 M 2-amino-2-methyl-1-propanol buffer at pH 9.6 and 25 °C. A spectrum of the chemiluminescence exhibited a maximum at 495 nm and was identical to the fluorescence spectrum of ester **4** under the same conditions. Chemiluminescence quantum yields were calculated from integration of total light output with a photon-counting luminometer and are relative to the known quantum yield of the chemiluminescent reaction of luminol. Table 2 shows a comparison of the chemiluminescent quantum yields of dioxetane **3a** compared to model dioxetane **1a**. In aqueous solution dioxetane **3a** is 5,000-fold more efficient than dioxetane **1a**. Expectedly, in DMSO the quantum yields are comparable.

Scheme 4. Chemical and Enzymatic Triggering of Dioxetanes **3a** and **3b**

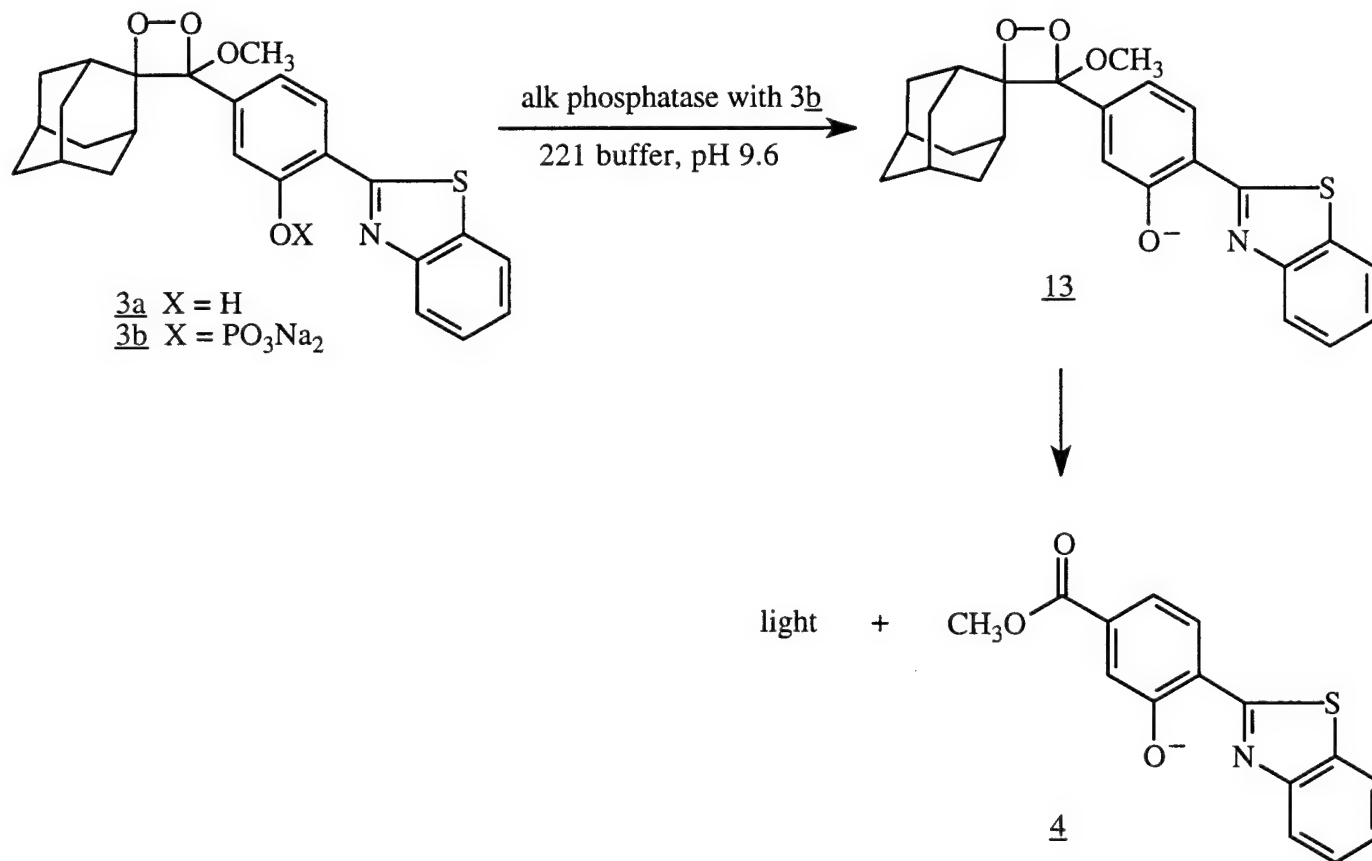


Table 2. Chemiluminescence Quantum Yields for Dioxetanes 3a and 1a

Dioxetane	$\lambda_{\text{max}}$	$\Phi_{\text{Cl}} (\times 10^{-2})$	Conditions
<u>3a</u>	483	5.0	aq. buffer
<u>3a</u>	495	21	DMSO / F <sup>-</sup>
<u>1a</u>	470	$8 \times 10^{-4}$	aq. buffer
<u>1a</u>	470	25	DMSO / F <sup>-</sup>

Enzymatic triggering of dioxetane 3b with alkaline phosphatase was investigated in 0.75 M 2-amino-2-methyl-1-propanol at pH 9.6 and 37 °C. Alkaline phosphatase from calf intestine was purchased from Biozyme Laboratories as a solution in 50% glycerol. A time profile for the chemiluminescence is shown in Figure 2. The luminescence increases to a maximum with time and reaches a plateau at which point the rate of enzymatic dephosphorylation is equal to the rate of decomposition of the unstable intermediate dioxetane 13. Various control experiments were conducted to verify that the chemiluminescence was the result of enzymatic hydrolysis of the phosphate group.

A chemiluminescence spectrum of dioxetane 3b triggered by alkaline phosphatase was also obtained. A small amount of enzyme was used to obtain constant light intensity. The resulting spectrum shown in Figure 3 is identical to the fluorescence spectrum of 4 under the same conditions. Both spectra exhibit maxima at 483 nm.

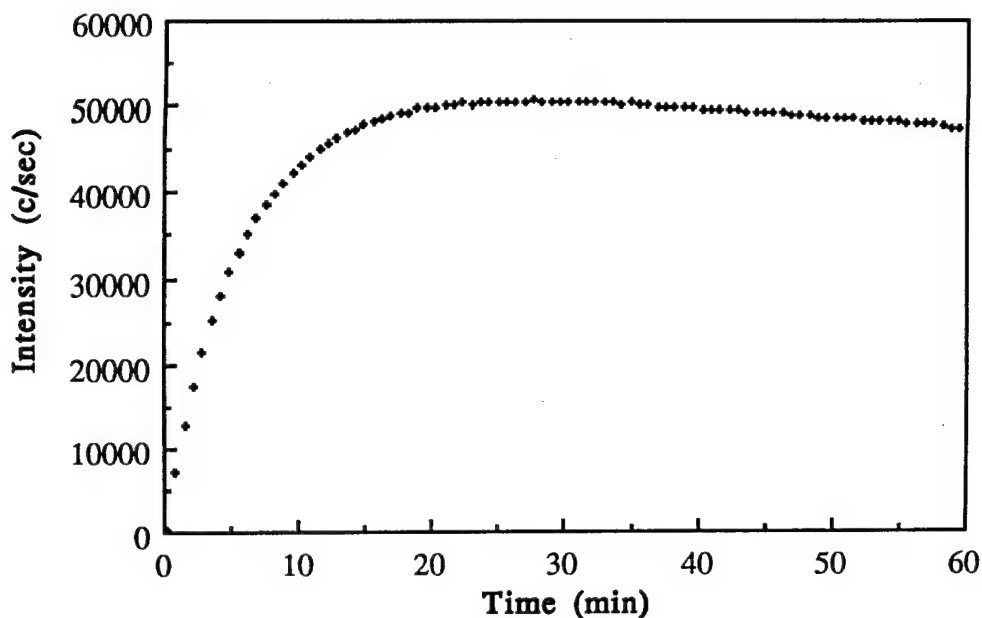


Figure 2. Time profile of the chemiluminescence of dioxetane 3b (0.32 mM) triggered by alkaline phosphatase ( $1.38 \times 10^{-12}$  M) in 0.75 M 2-amino-2-methyl-1-propanol buffer pH 9.6 and 37 °C

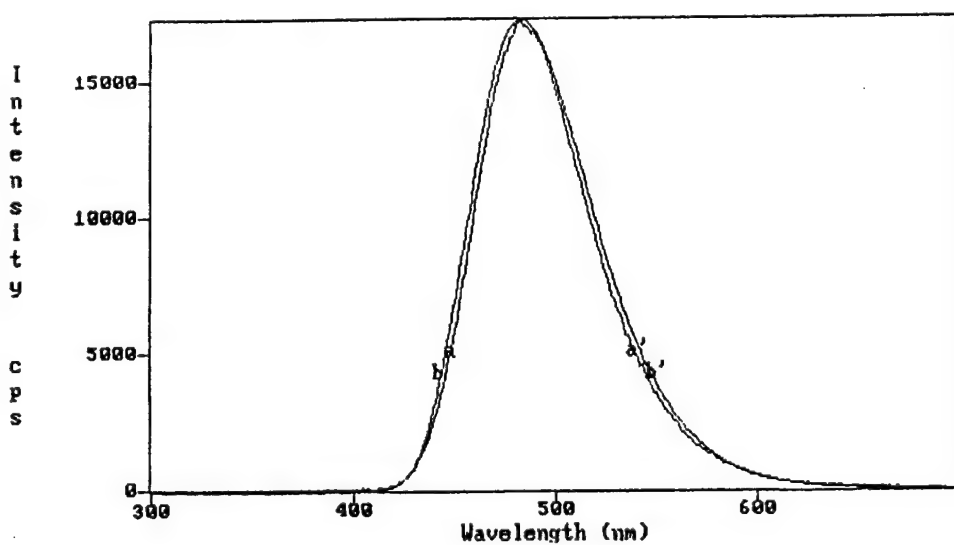


Figure 3. Chemiluminescence spectrum of dioxetane 3b triggered by alkaline phosphatase in 0.75 M 2-amino-2-methyl-1-propanol buffer pH 9.6 ( $\lambda_{\text{max}}=483$  nm, a-a') and fluorescence spectrum of 4 (b-b') under the same conditions.

## 2. Chemiluminescent Substrates for $\alpha$ -Chymotrypsin

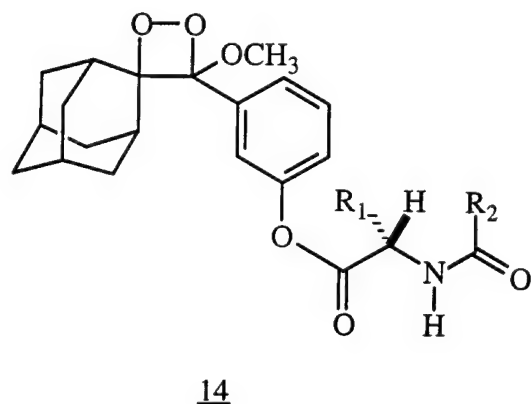
### Statement of Problem

The objective of this project was to develop chemiluminescent substrates for protease enzymes which could provide ultrasensitive assays for this class of enzymes in aqueous solution.

### Summary of Results

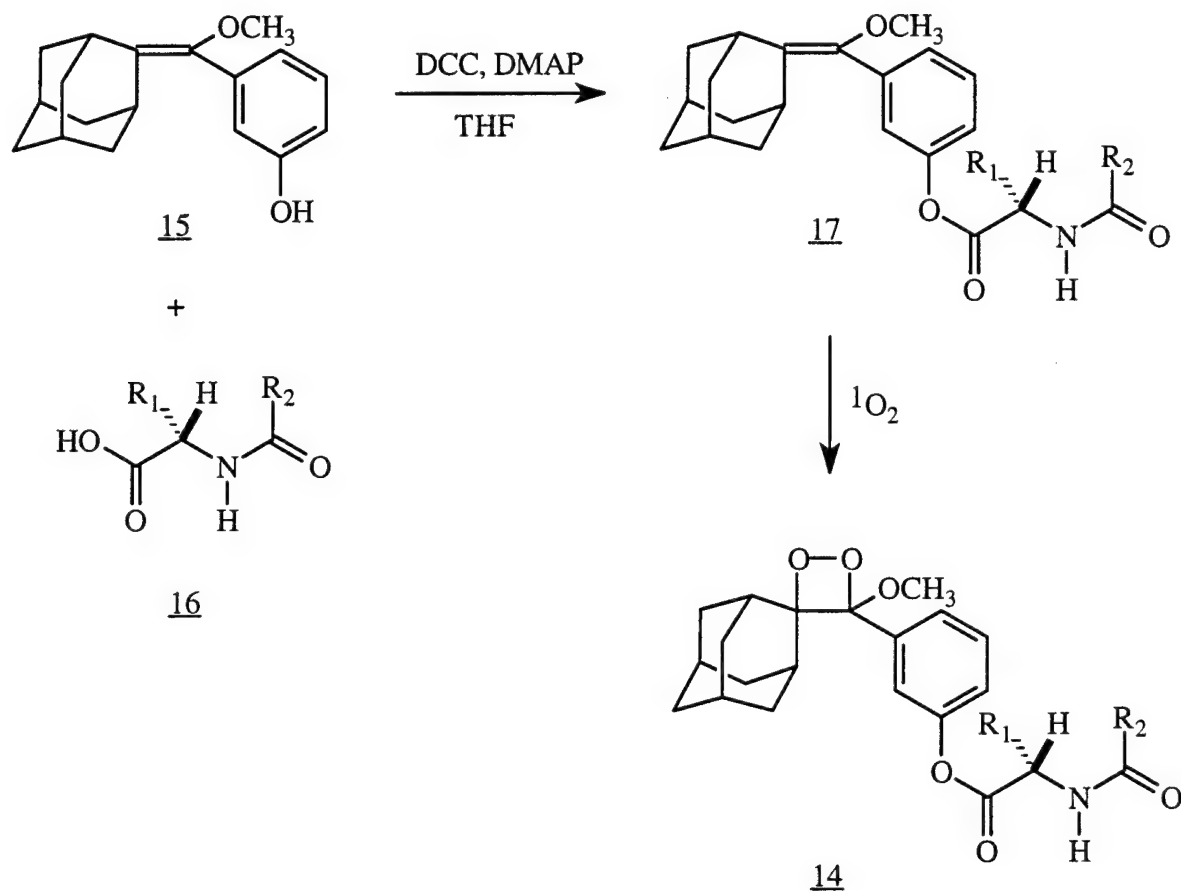
#### A. Synthesis of Dioxetanes 14a-e.

Dioxetanes 14a-e bearing an N-protected l-amino acid triggerable group were synthesized by the sequence of reactions shown in Scheme 5. The groups  $R_1$  in dioxetanes 14a-d are those found in phenylalanine, tyrosine and tryptophan, respectively.



	$R_1$	$R_2$
a		
b		CH <sub>3</sub>
c		CH <sub>3</sub>
d		CH <sub>3</sub>
e		CH <sub>3</sub>

Scheme 5. Synthesis of Dioxetanes 14a-e



After establishing that each of 14a-e were triggered by chymotrypsin, the chemiluminescence kinetics of 14a and 14b with  $\alpha$ -chymotrypsin were compared. Dioxetane 14b with the smaller acetyl N-protecting group was found to produce higher plateau intensities and reach the plateau more quickly at pH 8.5. The chemiluminescence spectrum produced in the reaction of chymotrypsin with 14b at pH 8.5 matched that of 1a under similar conditions with a  $\lambda_{\text{max}}$  at 470 nm. In a subsequent study, it was determined that a pH of 8.2 provided the optimum signal/background results in an enzyme assay due to the conflicting demands of substrate and enzyme stability on one hand and the need for a high pH to deprotonate the intermediate phenolic dioxetane. Running the assays at this relatively low pH came at the expense of slow rise to



maximum intensities, requiring > 20 minutes at 37 °C. The detection limit of  $\alpha$ -chymotrypsin using 14b was determined to be  $3 \times 10^{-12}$  moles of chymotrypsin or  $3.7 \times 10^{-3}$  units with a  $K_m$  for 14b of  $5.1 \times 10^{-4}$  M.

The performance of dioxetanes 14b, 14c and 14d were briefly compared at pH 8.2, 8.5 and 8.7. While signal/background decreased with increasing pH for all three, dioxetane 14b provided the best results at all pH values. Dioxetane 14e bearing a p-nitrobenzyl group showed optimum sensitivity when reacted with chymotrypsin at pH 8.0 but gave rise to relatively high background emission.

### **3. Effect of Leaving Group on Performance of Chemiluminescent Substrates for Carboxyl Esterase**

#### Statement of Problem

The objective of this project was to determine the effect of different enzymatically cleavable ester groups on the properties of chemiluminescent substrates for a protease enzyme.

#### Summary of Results

##### A. Synthesis of Dioxetanes 18a-d.

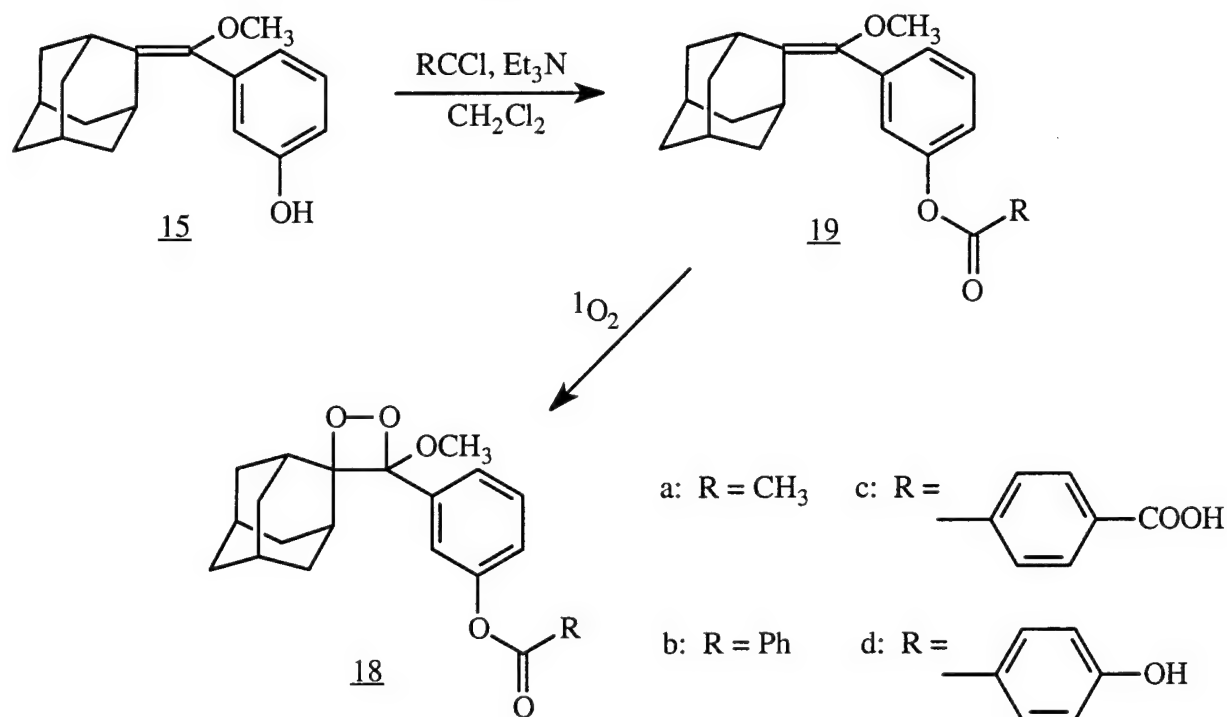
Dioxetanes 18a-d bearing different triggerable ester groups were synthesized from a common alkene intermediate followed by photooxygenation as shown below in Scheme 6.

##### B. Systematic Study of the Effect of the Cleavable Group by a Comparison of Dioxetanes 18 a-d

Having previously evaluated 18a as a chemiluminescent reagent for the detection of carboxyl esterase, we designed three new dioxetane compounds 18b, 18c and 18d, each having a different ester protecting group as potential esterase substrates. A systematic comparison allowed us to probe the effect of the ester moiety on hydrolytic stability, enzyme kinetics and chemiluminescence quantum yield. Reasoning that aryl esters would be hydrolyzed more slowly than alkyl esters, we

expected 18b to produce less background chemiluminescence than 18a under comparable conditions. This prediction was borne out; dioxetane 18b demonstrated better hydrolytic stability at pH 9.6. Enhancement of light emission with CTAB was judged impractical due to acceleration of hydrolysis.

Scheme 6. Synthesis of Dioxetanes 18a-d



Dioxetane 18c containing a negatively charged leaving group was evaluated to test whether the negative charge would impede attack of hydroxide at the ester group, particularly in the presence of CTAB. The hydrolytic stability of 18c was, however, inferior to that of 18b and was actually worsened by the inclusion of CTAB.

Dioxetane 18d bearing a  $\text{PhO}^-$  group, prepared to test whether a strongly electron-donating group might slow nucleophilic hydrolysis electronically, proved superior in hydrolytic stability in the presence or absence of CTAB.

### C. Determination of Enzyme Kinetic Parameters

Using a Lineweaver-Burk analysis,  $K_m$  for dioxetane 18d at 37 °C in pH 8.8 tris buffer was determined to be  $5.0 \times 10^{-6}$  M, indicating a high degree of affinity of the enzyme for the substrate. A small turnover number  $k_{cat}$  of 239 min<sup>-1</sup> or 4 s<sup>-1</sup> however limits analytical sensitivity. Nevertheless, the specificity constant  $k_{cat}/K_m$ ,  $8 \times 10^5$  M<sup>-1</sup>s<sup>-1</sup>, is comparable to other known substrates for this enzyme.

### D. Sensitivity of Assays for Carboxyl Esterase

The best sensitivity for detection of carboxyl esterase in a one-step assay was  $2 \times 10^{-13}$  moles using 18a and  $1 \times 10^{-13}$  moles using 18d. These results were obtained using a pH 8.8 reaction buffer. Since dioxetane 18d displayed acceptable stability in pH 9.6 221 buffer at 37 °C and chemiluminescence was markedly enhanced in the presence of CTAB, a two-step assay was devised. Enzyme incubation was performed at pH 8.1 for 15 minutes, then the pH was raised to 9.6 accompanied by the addition of CTAB and a fluorescein-linked surfactant in order to trigger light emission. A detection limit of  $3.2 \times 10^{-15}$  moles was achieved. This compares favorably with the results presented above using a dioxetane containing a tethered fluorescer. CTAB was incorporated in the high pH trigger solution since it was discovered to completely inhibit the enzyme, presumably due to enzyme denaturation.

While the sensitivity levels achieved are probably adequate for most purposes, targets for further improvements in assay sensitivity include developing substrates with which the enzyme can achieve higher catalytic rates of deacylation or improving the chemiluminescence quantum yield of the dioxetane under conditions which favor enzyme activity.

#### 4. Chemiluminescent Substrates for $\beta$ -Glucuronidase

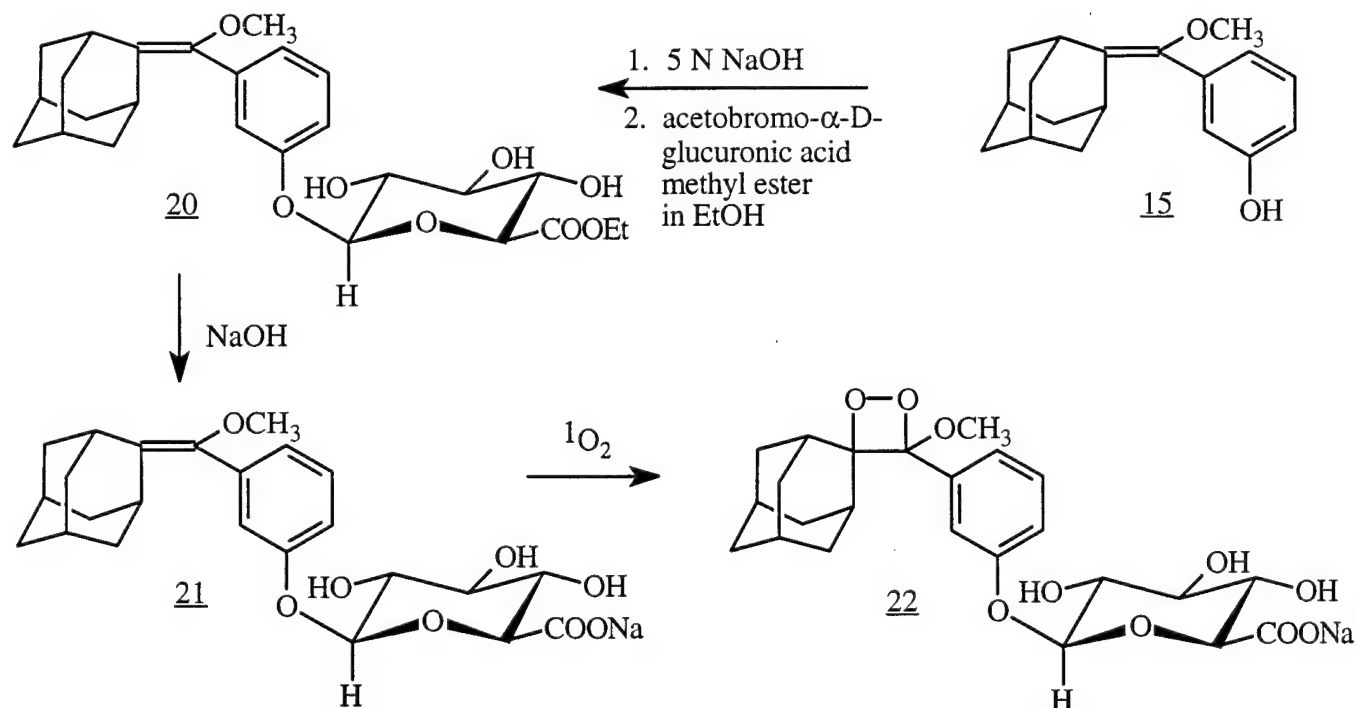
##### Statement of Problem

$\beta$ -Glucuronidase is widely used to detect the presence of *E. coli* as an indicator for the contamination of food and water. Further, this enzyme is also utilized in molecular biology research as a marker for gene fusion assays. Therefore, we elected to develop a chemiluminescent substrate for  $\beta$ -glucuronidase which incorporated the adamantyl meta-phenyl dioxetane structure.

##### Summary of Results

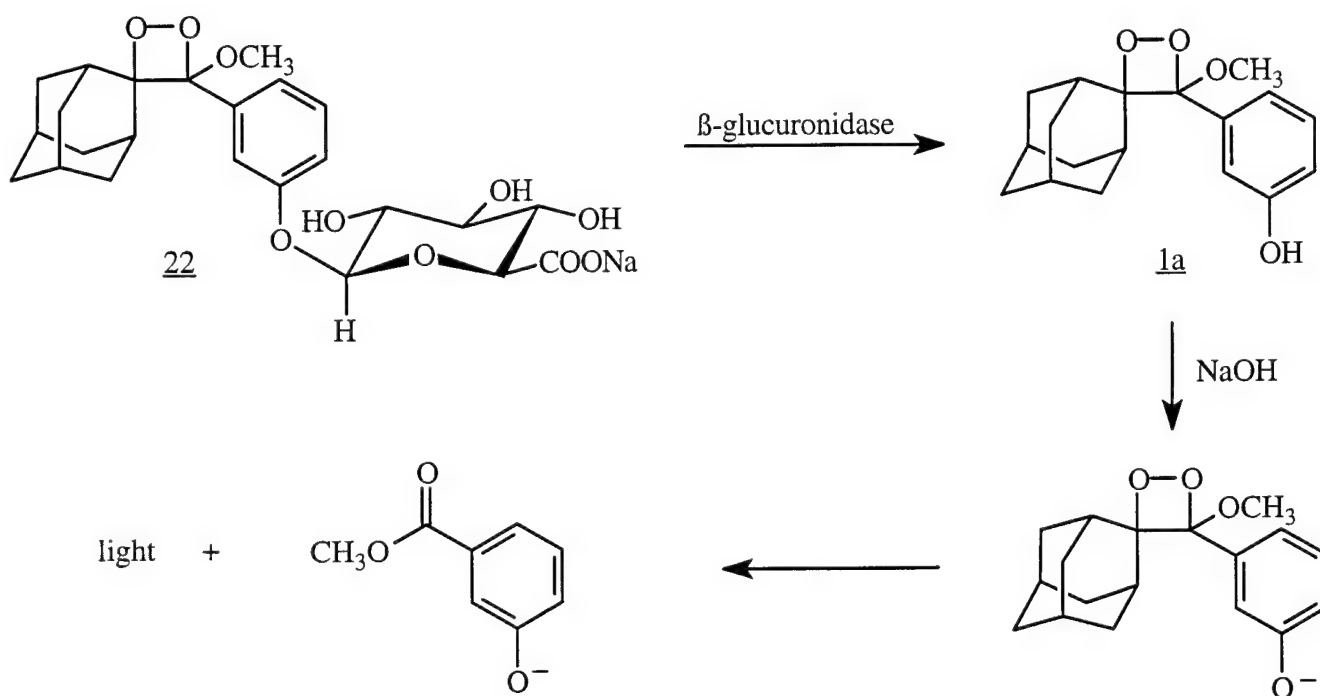
The glucuronide-substituted dioxetane 22 was synthesized as shown in Scheme 7. Hydroxyalkene 15 was treated with 5 N sodium hydroxide in acetone followed by addition of bromo-2,3,4-tri-*o*-acetyl- $\alpha$ -D-glucuronic acid methyl ester. Subsequent hydrolysis gave 21 which was photooxygenated at  $-78^\circ\text{C}$  to give dioxetane 22.

Scheme 7. Synthesis of Glucuronic Acid Phenyl Dioxetane 22.



Enzymatic triggering of dioxetane 22 was conducted in 2 steps. Removal of the glucuronide-protecting group with  $\beta$ -glucuronidase forms the hydroxyphenyl dioxetane 1a which accumulates under the conditions of the enzymatic reaction. Subsequent addition of alkali leads to deprotonation of the hydroxy group to generate the unstable phenoxide-substituted dioxetane which decomposes to produce chemiluminescence (Scheme 8).

Scheme 8.  $\beta$ -Glucuronidase-initiated Chemiluminescence from Dioxetane 22.



The glucuronidase triggering experiments were conducted in 0.1 M acetate buffer at pH 5.0. In a typical experiment 3 mL of acetate buffer containing  $\beta$ -glucuronidase ( $5.91 \times 10^{-9}$  moles) and 0.2% NaCl was equilibrated at 37 °C for 5 minutes. An aliquot of 150  $\mu\text{L}$  (52 mM) of a stock dioxetane solution (in methanol: p-dioxane 15:85 v/v) was added to the above solution and incubated for 20 minutes at 37 °C. After the incubation, 0.1 mL of 10 N NaOH solution was

injected giving rise to the chemiluminescence signal. The maximum intensity for the chemiluminescence profile was 800,000 cps (Figure 4). The conclusion that the chemiluminescence in the above experiment was caused by the enzymatic hydrolysis was verified by performing the above experiment in the absence of the enzyme.

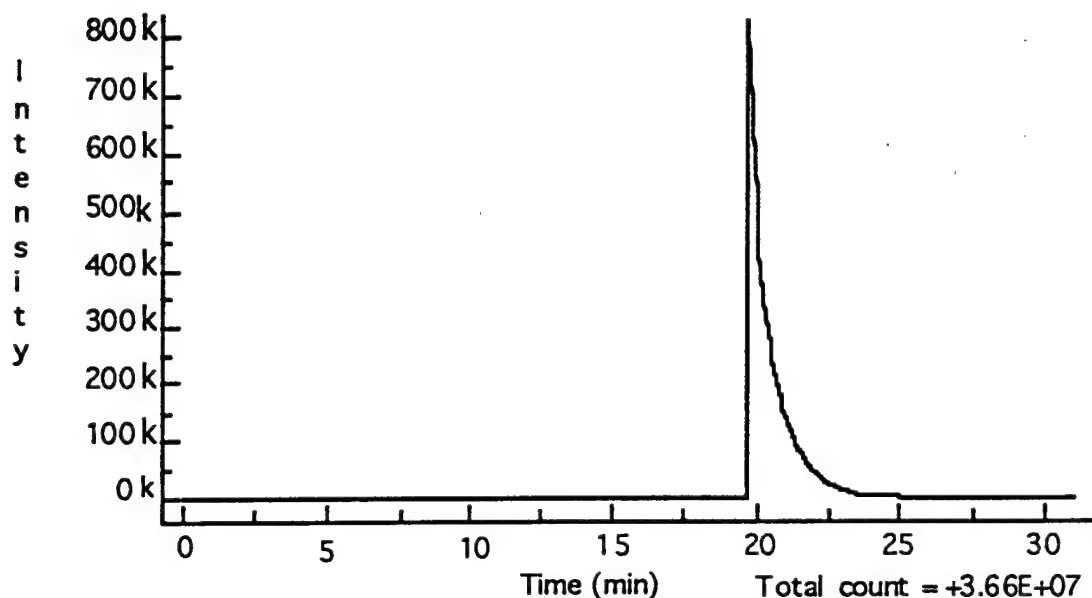


Figure 4. Plot of chemiluminescence produced by the treatment of dioxetane 22 with  $5.91 \times 10^{-9}$  moles of  $\beta$ -glucuronidase for a period of 20 min in 0.1 M acetate buffer (pH 5.0) at 37 °C followed by addition of NaOH.

$K_m$  for this substrate was determined by a series of concentration experiments. Lineweaver-Burk (Figure 5) and Eadie-Hoffstee (Figure 6) plots gave values for  $K_m$  of  $9.01 \times 10^{-4}$  M and  $8.76 \times 10^{-4}$  M, respectively. These plots are shown below. For comparison, phenolphthalein glucuronide has a  $K_m$  value of  $3.9 \times 10^{-4}$  M. The turnover number for dioxetane 22 with  $\beta$ -glucuronidase (Helix Promatia) was also determined as follows. Acetate buffer (3 mL, 0.1 M, pH

5.0) containing  $5.91 \times 10^{-9}$  moles of  $\beta$ -glucuronidase was thermally equilibrated at 37 °C for 5 min. Dioxetane 22 (150  $\mu$ L, 52 mM) was added to the above solution. After 20 minutes, 100  $\mu$ L of 10 N NaOH solution was injected into the solution through a light-tight rubber septum. The addition of strong base increased the solution pH to 12.0. As a result  $\beta$ -glucuronidase was inactivated and the rise in pH caused rapid decomposition of the hydroxy dioxetane liberated by the enzyme. The total light emitted during the enzymatic process and during the base induced reaction provides the direct measure of hydroxy dioxetane formed by the enzymatic hydrolysis of the substrate. The turnover number was found to be  $0.1 \text{ s}^{-1}$ .

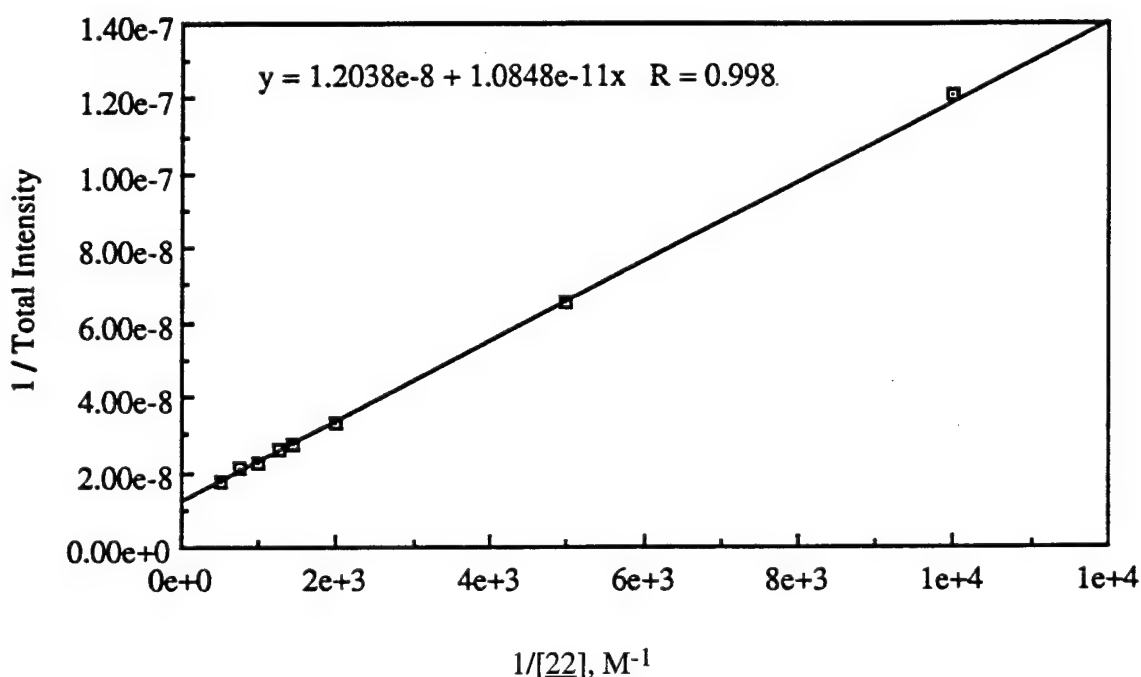


Figure 5. Lineweaver-Burk plot of the reciprocal of the concentration of 22 vs. the reciprocal of the total chemiluminescence intensity with *Helix Promatia*  $\beta$ -glucuronidase ( $5.91 \times 10^{-9}$  moles) in 0.1 M acetate buffer (pH 5.0) at 37 °C.

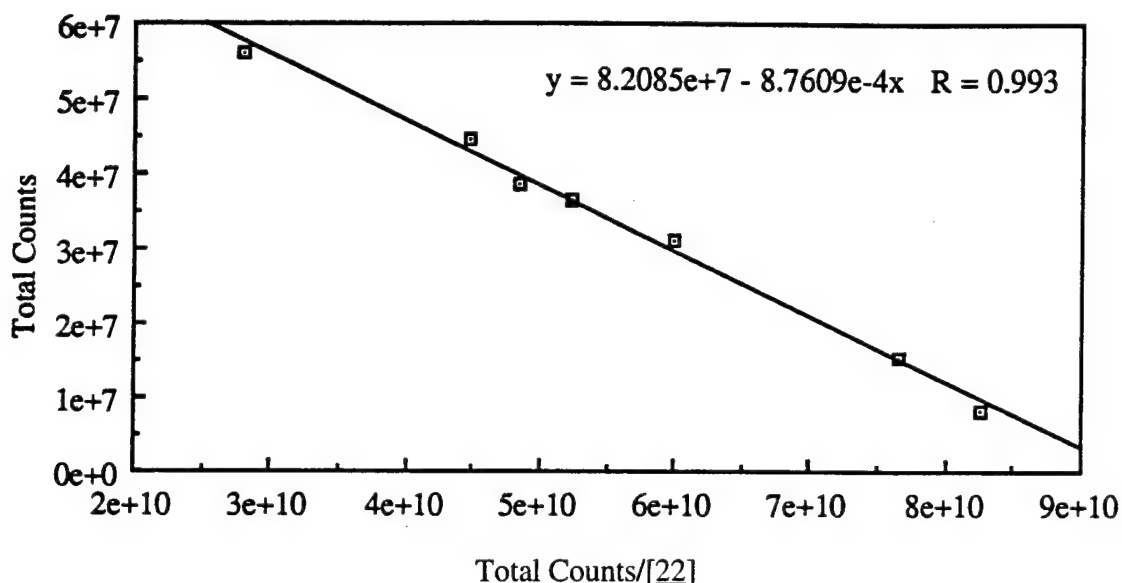


Figure 6. Eadie-Hoffstee plot for the determination of  $K_m$  of 22.

Assays for  $\beta$ -glucuronidase are used in the detection of *E.coli*, a food contaminant. Therefore, it was of interest to determine the detection limit of this enzyme obtained from *E.coli* using dioxetane 22 as a substrate.  $\beta$ -glucuronidase from *E.coli* was obtained from Sigma Chemical Company as lyophilized powder containing 31% protein. The commercial sample contained 8,800 units/mg. The enzyme solution was prepared in phosphate buffer at pH 6.8 containing 0.1% BSA. In a typical experiment 100  $\mu$ L of enzyme was added to 2.4 mL phosphate buffer. The solution was equilibrated at 37  $^{\circ}$ C for 5 min. Dioxetane 22 (210  $\mu$ L, 230 mM) was added to the above solution and incubated for 60 minutes. After the incubation period, 0.3 mL of phosphate buffer containing  $1.12 \times 10^{-2}$  M CTAB and  $6.4 \times 10^{-4}$  M fluorescein were injected followed by 100  $\mu$ L 10 N NaOH causing chemiluminescence signal. Similar experiments were performed by diluting the enzyme concentration. The plot of log enzyme concentration vs. log total intensity gave a straight line. Similarly, the background chemiluminescence was determined by performing the above experiment but without the enzyme. The lowest detectable amount of enzyme was 0.015 unit with signal to background ratio of 2. This amount corresponds to  $1.89 \times 10^{-9}$  g or  $7.57 \times 10^{-15}$  moles of  $\beta$ -glucuronidase per mL.



## 5. Chemiluminescent Substrate for Aryl Sulfatase

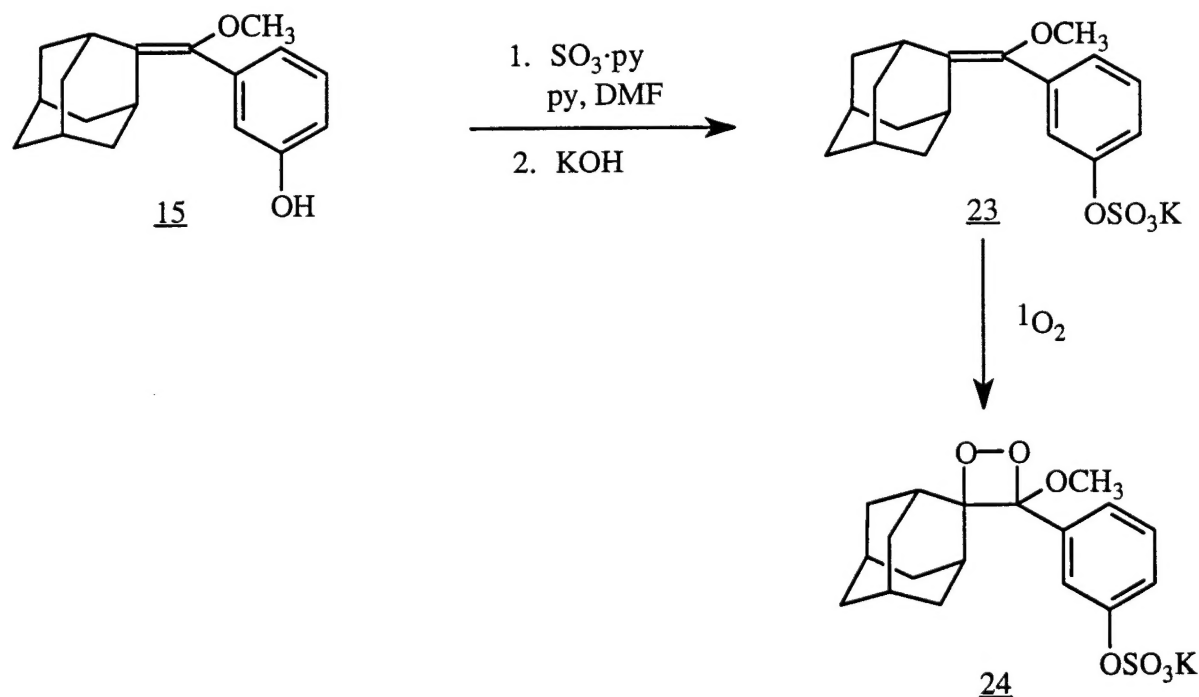
### Statement of Problem

The goal of this research project was to develop the first chemiluminescent substrate for the detection of aryl sulfatase.

### Summary of Results

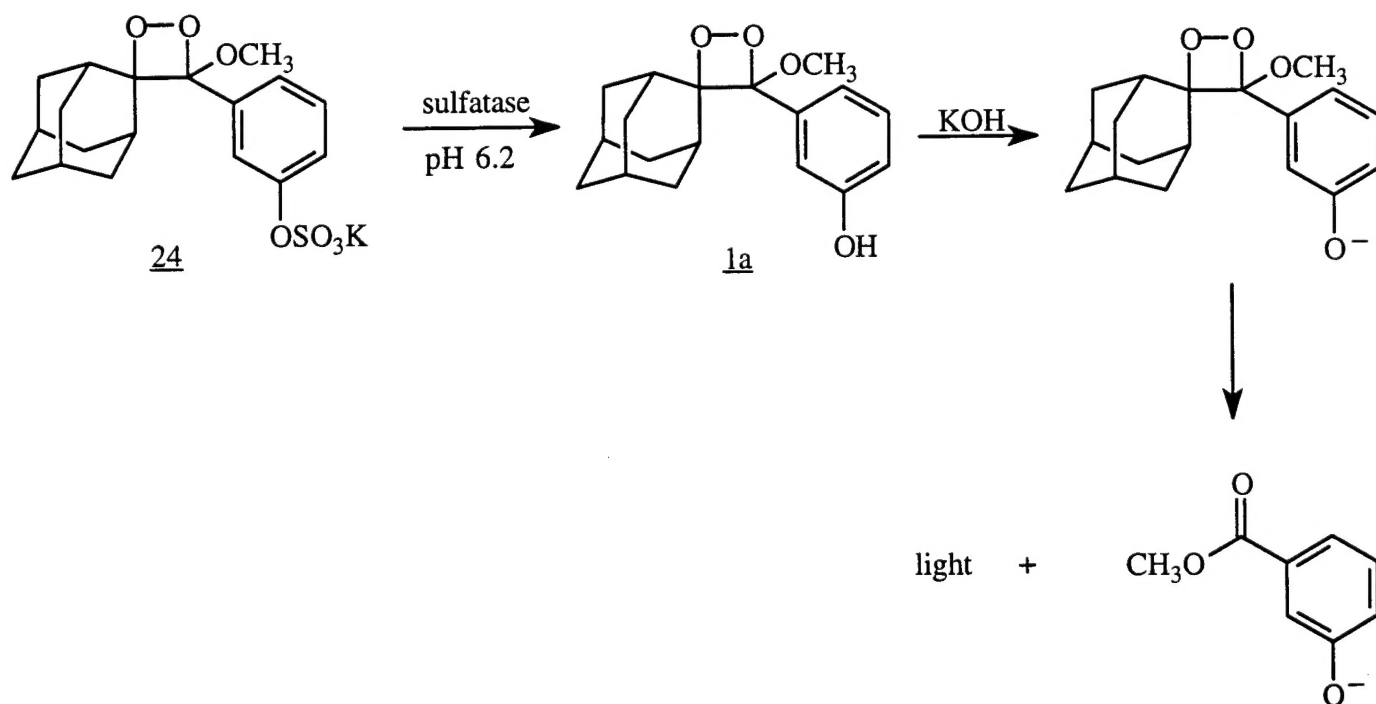
4-Methoxy-4-(3-sulfatephenyl)spiro[1,2-dioxetane-3,2'-adamantane] (24) was synthesized as shown in Scheme 9. Hydroxyphenyl alkene 15 was converted to the sulfate salt with 5 equivalents of pyridine-sulfur trioxide complex in anhydrous 2:1 DMF/pyridine. The addition of pyridine was essential to keep the vinyl ether from decomposing in the presence of sulfuric acid. The reaction was carried out overnight at 60 °C. Under such conditions, no sulfonation of the aromatic ring was detected. The resulting salt of the sulfate-substituted alkene 23 was quantitatively photooxygenated at low temperature in a 1:1 mixture of dioxane:water affording the sulfatephenyl dioxetane 24.

Scheme 9. Synthesis of Sulfatephenyl Dioxetane 24



Enzyme-induced cleavage of dioxetane 24 with arylsulfatase EC 3.1.6.1 from *Helix Promatia* was conducted at acidic pH (6.2). Under these conditions, enzymatic removal of the sulfate protecting group produces hydroxyphenyl dioxetane 1a which accumulates (Scheme 10). Upon subsequent addition of base, the enzyme is denatured and deprotonation of the hydroxyl group results in chemically induced luminescence. The sulfatephenyl dioxetane 24 itself is stable under alkaline conditions. Such triggering by addition of base after an incubation period induces the decomposition of the entire quantity of deprotected dioxetane 1a and delivers a short burst of light similar to that shown in Figure 2. The quantity of deprotected dioxetane 1a was found to increase linearly with the length of the incubation period. Experiments were also conducted in which the concentration of dioxetane 24 and aryl sulfatase were varied. The effects of pH and temperature were also studied. The use of fluorescent micelles to enhance the luminescence was also investigated.

Scheme 10. Enzymatic Triggering of Sulfatephenyl Dioxetane 24



## PARTICIPATING SCIENTIFIC PERSONNEL

### Graduate Research Assistants

<u>Name</u>	<u>Degree</u>	<u>Earned</u>
Batra, Moridu	M.A.	1995
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Dissanayake, Aruna	Ph.D.	1995
Josso, Martin	Ph.D.	1992
Ladonchamps, Aurelie	M.S.	1990
Thakur, Kumar	M.S.	1993

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## REFERENCES

The following Wayne State University Ph.D. dissertations and M. S. theses are gratefully acknowledged. Additional information concerning this research and citations to the appropriate literature are found in these documents. The projects described herein were also supported in part by the National Science Foundation and Wayne State University.

Aruna S. Dissanayake, 1995, Ph.D. Dissertation

*Efficient Chemiluminescence from 4-(4-Benzothiazoyl-3-hydroxyphenyl)-4-methoxyspiro(1,2-dioxetane-3,2'-adamantane) and Derivatives*

Martin Josso, 1992, Ph.D. Dissertation

*I. Development of Chemiluminescent Assays for  $\alpha$ -Chymotrypsin and Carboxylesterase.*

*II. Efficient Chemiluminescence from a 1,2-Dioxetane Initiated by Pyranine.*

Aurelie Lefebvre De Ladonchamps, 1990, M.S. Thesis

*I. Arylsulfatase-Induced Chemiluminescence from 4-Methoxy-4-(3-sulfatephenyl) spiro[1,2-dioxetane-3,2'-adamantane]. II. Base-Induced Chemiluminescence from 4-(2-chloro-5-hydroxyphenyl)-4-methoxyspiro[1,2-dioxetane-3,2'-adamantane].*

Kumar DattatrayaThakur, 1993, M.S. Thesis

*Development of a Chemiluminescent Assay for Beta-Glucuronidase*